

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| (51) International Patent Classification ⁵ : A61K 9/22 | A1 | (11) International Publication Number: WO 91/05548 (43) International Publication Date: 2 May 1991 (02.05.91) |
| (21) International Application Number: PCT/US90/05345 (22) International Filing Date: 20 September 1990 (20.09.90) (30) Priority data: 420,156 10 October 1989 (10.10.89) US (71) Applicant: PITMAN-MOORE, INC. [US/US]; 1401 S. Third Street, P.O. Box 207, Terre Haute, IN 47808 (US). (72) Inventors: SIVARAMAKRISHNAN, Kallidaikurichi, N. ; 540 East 42nd Drive, Terre Haute, IN 47802 (US). GRAY, Matthew, W. ; R.R. #53, Box 515, Terre Haute, IN 47805 (US). | | (74) Agents: FIGG, E., Anthony et al.; Bernard, Rothwell & Brown, 1700 K Street, N.W. #800, Washington, DC 20006 (US). (81) Designated States: AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH (European patent), CM (OAPI patent), DE (European patent)*, DK (European patent), ES (European patent), FI, FR (European patent), GA (OAPI patent), GB (European patent), HU, IT (European patent), JP, KP, KR, LK, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL (European patent), NO, RO, SD, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent). Published <i>With international search report.</i> |
| (54) Title: SUSTAINED RELEASE COMPOSITION FOR MACROMOLECULAR PROTEINS (57) Abstract A sustained release composition for delivering macromolecular proteins to an animal over a prolonged period which comprises a solid wax matrix having the macromolecular protein and a water-insoluble surfactant uniformly dispersed therein is disclosed. The water-insoluble surfactant provides advantageous sustained release properties for the protein; the protein is released from the composition in amounts greater than amounts released from protein-wax compositions or from protein-wax compositions containing a water-soluble surfactant. | | |

ATTORNEY DOCKET NUMBER: 10271-007-999
SERIAL NUMBER: 09/724,396
REFERENCE: **AZ**

* See back of page

DESIGNATIONS OF "DE"

Until further notice, any designation of "DE" in any international application whose international filing date is prior to October 3, 1990, shall have effect in the territory of the Federal Republic of Germany with the exception of the territory of the former German Democratic Republic.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|---------------------------------------|----|--------------------------|
| AT | Austria | ES | Spain | MG | Madagascar |
| AU | Australia | FI | Finland | ML | Mali |
| BB | Barbados | FR | France | MR | Mauritania |
| BE | Belgium | GA | Gabon | MW | Malawi |
| BF | Burkina Faso | GB | United Kingdom | NL | Netherlands |
| BG | Bulgaria | GR | Greece | NO | Norway |
| BJ | Benin | HU | Hungary | PL | Poland |
| BR | Brazil | IT | Italy | RO | Romania |
| CA | Canada | JP | Japan | SD | Sudan |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SE | Sweden |
| CG | Congo | KR | Republic of Korea | SN | Senegal |
| CH | Switzerland | LI | Liechtenstein | SU | Soviet Union |
| CI | Côte d'Ivoire | LK | Sri Lanka | TD | Chad |
| CM | Cameroon | LU | Luxembourg | TG | Togo |
| DE | Germany | MC | Monaco | US | United States of America |
| DK | Denmark | | | | |

Sustained Release Composition for Macromolecular Proteins

MACROMOLECULAR PROTEINS

5 This invention relates generally to sustained
release compositions and particularly to a sustained
release composition for the prolonged release of
macromolecular proteins.

Background of the Invention

10 The delivery of macromolecular proteins to animals
is complicated by the need for delivery devices which
will maintain the bioactivity of the protein prior to,
during, and after administration to the animal. Merely
15 exposing macromolecular proteins to aqueous
environments causes the large proteins to form
precipitates and aggregates which destroy the
bioactivity and therefore the usefulness of the
protein. Also, hydrolytic reactions resulting from
20 "wetting" the proteins may produce bio-inactive
products which reduce the in vivo effectiveness of the
protein.

Prior art methods for overcoming this problem have
included encapsulating the macromolecular proteins in
polymer-coated tablets which limit the ingress of
25 water, pressing the macromolecular proteins into pellet
compositions which control the release of the protein
by limiting the surface area of the pellet and

therefore the amount of water that has access to the protein, placing the macromolecular proteins in porous devices which limit the ability of water to enter the device and the ability of the protein to exit the device, and the like. Patents and other references describing these methods are voluminous and well known in the art.

Sustained release of drugs can be achieved by dispersing or dissolving them in a water-insoluble matrix. Suitable matrix materials for such diffusion-controlled delivery devices include polymers such as poly(ethylene-co-vinyl acetate) (EVA), ethyl cellulose, and polysiloxanes. The mechanism of release is the slow diffusion of the dissolved drug through interconnecting pores and channels formed in the matrix. See, Rhine et al., J. Pharm. Sci., Vol. 69(3), p. 265 (1980) and Goodhart et al., J. Pharm. Sci., Vol. 63(11), p. 1748 (1974). The release characteristics of these devices are determined by the physical properties such as visco-elasticity, glass transition temperature, and porosity of the matrix.

Waxes have been used in solid dosage forms as lubricants, coatings, and inert fillers. Appropriate formulation of the drug in a wax matrix can give rise to sustained release of the drug. A sustained release dosage form of tripeleminamine hydrochloride was developed using carnauba wax as the matrix; Dakkuri et al., J. Pharm. Sci., Vol. 67(3), p. 354 (1978). The drug was dispersed along with a surfactant in molten carnauba wax containing stearyl alcohol. The congealed mass was granulated and compressed into cores. Hydrophobic surfactants such as glycerol monostearate did not affect the dissolution rate of the tablets. On the other hand, hydrophilic surfactants such as

polyoxyethylene lauryl ether had a profound effect, releasing about 80% of the drug in about 4 hours. The data were interpreted in terms of facile wetting of the matrix, promoted by the surfactant, thus creating
5 channels for the diffusion of the drug. Inclusion of polyvinylpyrrolidone in the wax matrix accomplished the same result.

Controlled delivery of indomethacin was achieved from carnauba wax matrix containing surfactants. A
10 combination of erosion, leaching, and solubilization of the drug was used to explain the sustained release of sulfaethylthiadiazole from tablets made by compressing a spray-congealed wax matrix; Hamid et al., J. Pharm. Sci., Vol. 59 (4), p. 511 (1970). Sustained release of
15 drugs could also be obtained through "slowly disintegrating tablets" as detailed in the U.S. Patent 4,695,467. Sustained elevation of plasma growth hormone level from a soybean oil/beeswax implant has been reported; Davis et al., J. Dairy Sci., Vol. 66, p.
20 1980-2 (1983).

Also, U.S. Patent No. 4,404,183 discloses a sustained release composition of solid medical material which has water-soluble surfactants such as polyethylene glycol as a component. U.S. Patent No.
25 4,404,183 discloses a controlled release composition which may contain surfactants which are soluble in water and in solvent and are stable at high temperatures. Neither of these patents, however, discloses the use of a water-insoluble surfactant.

30 Schroeder et al., J. Pharm. Sci., Vol. 67, p. 350 (1978) discloses sustained release combinations of small organic drugs such as tripeleminamine hydrochloride or tolazoline hydrochloride from wax matrixes. Similarly, Hamid et al., J. Pharm. Sci.,

Vol. 59 (4), p. 511 (1970) discloses sustained release compositions containing a small organic drug sulfaethylthiadiazole in wax matrixes. The compositions did not, however, contain surfactants nor provide release characteristics for macromolecular proteins.

Al-shora et al., Int. J. Pharm., Vol. 7, p. 77 (1980) discloses using wax matrixes for producing sustained release compositions containing small organic drugs such as tripeleennamine hydrochloride and water-soluble channeling agents such as polyethylene glycol. The compositions did not, however, contain water-insoluble surfactants nor provide a sustained release composition for macromolecular proteins.

Because of the problems associated with prior sustained release compositions and devices for macromolecular proteins, there exists a continuing need for new sustained release compositions which control the manner and timing of delivery while maintaining the stability and bioactivity of the macromolecular proteins when the device is administered to an animal.

Summary of the Invention

It is, therefore, an object of the present invention to provide a sustained release composition for macromolecular proteins.

It is another object of the present invention to provide a sustained release composition for macromolecular proteins which controls the manner and timing of delivery while maintaining the stability and bioactivity of the macromolecular proteins.

These and other objects are achieved using a sustained release composition for macromolecular proteins which comprises a solid wax matrix having the

macromolecular protein and a water-insoluble surfactant uniformly dispersed therein. The water-insoluble surfactant provides advantageous sustained release properties for the protein; the protein is released from the composition in amounts greater than amounts released from protein-wax compositions or from protein-wax compositions containing a water-soluble surfactant. The total amount of protein released from the present composition is greater than the total amount of protein released from protein-wax compositions or from protein-wax compositions containing a water-soluble surfactant and the amount of protein released at any particular time following administration is greater than the amount of protein released from protein-wax compositions or from protein-wax compositions containing a water-soluble surfactant.

Other objects, advantages, and novel features of the present invention will become apparent from the following detailed description of the invention.

Description of the Drawings

Figure 1 shows the cumulative percent protein released for different weight percents of beeswax compositions containing Mazol®.

Figure 2 shows the cumulative percent protein released for different weight percents of beeswax compositions containing polypropylene glycol (PPG).

Figure 3 shows the cumulative percent protein released for different weight percents of beeswax compositions alone, containing PPG, and containing pentaerytherol.

Figure 4 shows the cumulative percent protein released for different surfactants having different Hydrophile Lipophile Balance's (HLBs).

5 Figure 5 shows the cumulative percent lysozyme released for different Mazol® concentrations.

Figure 6 shows the cumulative percent Bovine Serum Albumin (BSA) released for different waxes.

Figure 7 shows the cumulative percent protein released for different formulation techniques.

10

Detailed Description of the Invention

The present invention is a sustained release composition for delivering macromolecular proteins to an animal over a prolonged period which comprises a
15 solid wax matrix having the macromolecular protein and a water-insoluble surfactant uniformly dispersed therein. Surprisingly, the inclusion of a water-insoluble surfactant in a wax-protein composition provides advantageous sustained release properties for
20 the protein; the protein is released from the composition in amounts greater than amounts released from protein-wax compositions or from protein-wax compositions containing a water-soluble surfactant. The total amount of protein released from the present
25 composition is greater than the total amount of protein released from protein-wax compositions or from protein-wax compositions containing a water-soluble surfactant, and the amount of protein released at any particular time following administration is greater
30 than the amount of protein released from protein-wax compositions or from protein-wax compositions containing a water-soluble surfactant.

The composition of the present invention can be formed by any procedure (1) which provides an

essentially uniform solid wax matrix containing the macromolecular protein and water-insoluble surfactant and (2) which is non-destructive to the macromolecular protein. Several such methods are known in the art.

5 In one preferred embodiment, (1) the wax is heated until it melts; (2) the water-insoluble surfactant is added to the melt and the surfactant and wax composition are mixed thoroughly until the surfactant is uniformly dispersed in the wax composition; and
10 (3) the macromolecular protein is added to the melt and the protein and wax are mixed thoroughly until the protein is uniformly dispersed in the wax. The order of addition of the protein and surfactant is not crucial.

15 In another embodiment, the dry particles of the wax, macromolecular protein, and water-insoluble surfactant are mixed thoroughly to produce a homogeneous mixture. The mixture is subsequently compressed or preferably molded into the desired size
20 and shape.

Waxes useful for producing the composition include animal waxes such as beeswax, lanolin, shellac wax, and Chinese insect wax; vegetable waxes such as hydrogenated soybean oil, cottonseed oil, carnauba,
25 candelilla, bayberry, and sugar cane; and mineral waxes such as fossil or earth waxes (ozocerite, ceresin, montan) and petroleum waxes (paraffin, microcrystalline, slack or scale wax), or combinations thereof. Preferably, the wax material used in the
30 present invention is beeswax, vegetable wax, carnauba wax, or combinations thereof.

The wax or combination of waxes should comprise from about 50-99% by weight of the composition, preferably from about 70-99%.

Macromolecular proteins suitable for inclusion and thus deliverable in the sustained release composition of the present invention include but are not limited to proteins having a molecular weight from about 2000 to about 200,000 daltons. The macromolecular proteins include but are not limited to natural, recombinant, synthetic and mutein proteins which have deleted, inserted, substituted, or otherwise modified sequences and biologically active fragments and derivatives thereof. More specifically, bioactive proteins such as enzymes, enzyme inhibitors, antibodies, antigens, interferons, insulins, prolactins, somatomedins, somatostatins, interleukins, somatocrinins (GRF) and somatotropins can be delivered according to the present invention.

Preferably, human, porcine, bovine, equine, ovine, and avian somatotropins can be delivered using the sustained release compositions of the present invention. Somatotropin is defined herein to include all proteins having somatotropin activity including the natural, recombinant, synthetic, and mutein somatotropins having deleted, inserted, substituted, or otherwise modified sequences and biologically active fragments and derivatives thereof. Additionally, metals or metal compounds associated with biologically active proteins, peptides and polypeptides, as well as acid salts, derivatives and complexes and antihydrating agents are suitable for incorporation into the sustained release composition of the invention.

Somatotropins useful in the present invention can be obtained from any suitable source. Methods for producing, isolating and purifying native and recombinant somatotropins are well known in the art. The amino acid sequences of various somatotropins

useful in the present invention are well known: C.H. Li in Kirk-Othmer "Encyclopedia of Chemical Technology", 3rd E., Vol. 12, pp. 549-552 (human somatotropin), R.P. Woychik, Nucleic Acid Res., Vol. 10, p. 7197 (1982)
5 (bovine somatotropin), C.H. Li et al., Arch. Biochem. Biophys., Vol. 156, p. 493-508 (1973) (ovine somatotropin) and P.H. Seeburg et al., DNA, Vol. 2, p. 37, 45 (1983) (porcine somatotropin).

Recombinant somatotropins are also known in the
10 art. European Patent Application Publication No. 0 103 395 describes the construction of a transformant strain of E. coli containing a first plasmid which codes for delta-9 (Ser) bovine somatotropin (somatotropin less its 9 N-terminal amino acids and having an additional
15 serine residue at the N-terminus) under the control of the lambda P_L promoter-operator and which has a Shine-Dalgarno region derived from bacteriophage mu. The transformant also contains a second plasmid, pcI857, which codes for the production of the pcI857
20 temperature-sensitive repressor protein. The repressor protein can be inactivated by raising the temperature to about 42°C, thereby inducing expression of delta-9 (Ser) bovine somatotropin. A transformant strain of this type, E. coli HB101 (P_L-mu-delta-9 (Ser) bovine
25 somatotropin and pcI857) has been deposited with The American Type Culture Collection (ATCC), Rockville, MD, and assigned Accession No. 53030.

Construction of a similar transformant strain which codes for the production of delta-7 porcine
30 somatotropin (porcine somatotropin less its first 7 N-terminal amino acids) is described in European Patent Application Publication No. 0 104 920. A transformant strain of this type, E. coli HB101 (P_L-mu-delta-7

porcine somatotropin and pcI857) has been deposited with ATCC and assigned Accession No. 53031.

Strains 53030 and 53031 are prolific producers of delta-9 (Ser) bovine somatotropin and delta-7 porcine somatotropin, respectively. Other methods for many similar proteins are known in the art.

The macromolecular proteins should comprise about 1-30% by weight of the composition, preferably from about 5-20%.

Water-insoluble surfactants suitable for inclusion in the sustained release composition of the present invention include non-ionic, water-insoluble surfactants having a molecular weight (MW) of from about 100-2000 and a Hydrophile Lipophile balance (HLB) value of from about 1-17. Such surfactants include glycerol and polyglycerol esters, fatty alcohol ethers, ethoxylated sorbitan fatty acid esters, and the like. Examples of such surfactants, described generally in Table 1, include but are not limited to Mazol®, Macol® CA-2, Macol® CA-20 and T-MAZ®-61. Preferably, the surfactant used in the present invention is Mazol® 80 MG-K. The composition and characteristics of these surfactants are well known to skilled artisans. For example, the surfactants used herein can be purchased from Mazer Chemicals, a division of PPG Industries Chemical Group, Gurnee IL.

The surfactant should comprise from about 0.1-20% by weight of the composition, preferably from about 0.5-10%.

In the preferred embodiment of the present invention, the composition comprises from about 50-99% beeswax, 1-30% somatotropin, and 0.1-20% Mazol®.

The composition of the present invention can be produced in many shapes and sizes. The wax melt or dry

mixture containing the protein and surfactant can be molded and shaped into any desired form and size. For example, the composition can be sliced into "slabs", shaped into pellets, rods or tablets, and the like.

5 Also, the viscous wax melt can be poured into molds or the dry mixture can be compressed to produce implant devices which can be implanted into an animal.

Pellets, implant devices or tablets preferably comprise from about 50-99% wax, 1-30% protein and
10 0.1-20% water-insoluble surfactant. Such pellets, implant devices or tablets are designed to deliver the protein to the animal in the required amounts, typically from about 0.1-20 mg protein/animal/day, over a prolonged period, typically from about 1-14 days.

15 Preferably, pellets, implant devices or tablets comprise from about 50-99% beeswax, 1-30% somatotropin and 0.1-20% Mazol®. Such pellets, implant devices or tablets are designed to deliver from about 0.1-20 mg somatotropin/animal/day, preferably from about 1-10
20 mg/animal/day, over a period of from about 1-14 days. The resulting pellet, implant device or tablet can be implanted into an animal to promote growth and increase feed utilization efficiency.

According to the present invention, a method for
25 delivering macromolecular proteins to an animal comprises administering to the animal the sustained release composition of the present invention.

In another aspect of the present invention, a method for promoting growth and increasing feed
30 utilization efficiency in an animal over a prolonged period comprises administering to an animal the device of the present invention containing somatotropin as the macromolecular protein. The somatotropin should be present in the sustained release composition in an

amount effective to promote growth when administered to the animal. Although the amount of somatotropin required to promote growth may vary depending upon the particular somatotropin, the type of animal, and the
5 desired results, the somatotropin is generally present in amounts from about 1-30% by weight of the composition, preferably from about 5-20%. Somatotropin is typically administered to animals in dosages from about 0.1-20 mg/animal/day, preferably from about 1-10
10 mg/animal/day to promote growth and increase feed utilization efficiency.

The invention having been generally described, the following examples are given as particular embodiments of the invention and to demonstrate the practice and
15 advantages thereof. It is understood that the examples are given by way of illustration and are not intended to limit the specification or the claims to follow in any manner.

Materials used in the Examples

20 The following materials and equipment were used in the examples herein: Bovine Serum Albumin (BSA), lysozyme and micrococcus lysodekticus were obtained from Sigma Chemical Co., St. Louis, MO. Lysozyme was purified, sterilized, and lyophilized before use.

25 Beeswax (white bleached) and candelilla wax (#1 Yellow American) were obtained from Strahl & Pitsch Inc., West Babylon, NY. Stearine (Durkee-07) was obtained from Durkee Industrial Foods, SCM Corporation, Cleveland, Ohio.

30 Surfactants Mazol® 80 MG-K, Macol® CA-2, Macol® CA-20 and T-MAZ®-61 were obtained from Mazer Chemical Co., Gurnee, IL. The properties of these surfactants are listed in Table 1. Polypropylene glycol (PPG;

molecular weight: 425 daltons) was obtained from Aldrich Chemical Co., Milwaukee, WI. Pentaerythritol was sieved to less than 106 microns. A Carver laboratory press (Model #2698 made by Carver Inc., Menomonee Falls, WI.) was used to prepare tablets. Release studies were conducted by placing the slabs of tablets in test tubes and shaking them with the buffer solution in a water shaker bath at 37°C. UV absorbance was measured with a Perkin Elmer UV/VIS Spectrophotometer (Model: Lambda 7).

Methods used in the Examples

Sieving of Proteins: BSA was sieved to <106 microns and used in all the experiments except those which studied the effect of particle size on release profile (Trial 7). For Trial 7, BSA was used after sieving to the following fractions: 425-250 microns, 250-150 microns, 150-106 microns, 106-53 microns, and less than 53 microns. Purified lysozyme was sieved to 150-106 microns.

Preparation of wax/protein slabs: Beeswax and an appropriate amount of the additive (PPG, Mazol®, etc.) were placed in a 100 ml beaker and heated for about 2 hours in an oven at 150°C with occasional stirring. Five grams of the wax-additive mixture were transferred to a 10 ml beaker and equilibrated for an hour at 85°C in the heating block. The 10 ml beaker containing the wax mixture was then placed on a hot plate stirrer (kept at low setting to minimize heat loss). The protein (0.555 g) was added to the wax mixture, stirred at first with a spatula and then with a magnetic stir bar. The beaker was then placed back into the heating block for about 1 minute to allow wax on the sides of the beaker to remelt. The beaker was placed back onto

the hot plate and stirred briefly to resuspend the protein. The magnetic stir bar was removed and the wax/protein suspension was quickly transferred to an aluminum weighing pan (previously warmed on a hot plate). A spatula was used to quickly scrape the wax from the sides of the beaker into the pan. The pan was then transferred to a leveled thermoelectric cold plate set on the coldest setting. After the wax had solidified, the pan was removed from the cold plate and brought to room temperature. The procedure, from the addition of the protein to the wax to the initial solidification of the wax, took about 2 minutes. When pentaerythritol was used as the additive, it was dry-mixed with the solid protein before being added to the molten wax.

After cooling to room temperature, the slab of protein/wax matrix was removed from the pan and placed onto a glass plate. A rectangular section (2.0 cm x 4 cm) was cut from the center portion of the slab with a razor blade. This was further cut into eight smaller slabs measuring 2.0 cm x 0.5 cm x 0.20-0.25 cm (l x w x h). The smaller slabs were weighed individually prior to use in release studies. The loading of the protein was about 10% w/w.

Preparation of Compressed Wax Disks: Candelilla wax and Macol® CA-20 (100/2 by weight) were placed into a beaker and melted at 150°C for 1 hour to allow any trapped water to escape. The wax was poured onto a piece of aluminum foil, cooled and ground in a high speed grinding mill in intervals of 15 seconds. The wax powder was sieved after each interval to avoid undersizing the wax. The fraction from 250-150 microns was used.

"Dry-mixed" formulations were prepared by mixing BSA with Macol® CA-20 wax powder as prepared above in the ratio of 10/90 (w/w). The matrix (200 mg) was pelleted in a mold (Perkin Elmer; Dia: 1.3 cm) at
5 24,000 psi in the Carver Press.

"Melt-mixed" formulations were prepared by melting the candelilla wax with the Macol® CA-20 (2%) at 150C for one hour. Five grams of this mixture were placed into a beaker and cooled to 85°C and mixed with BSA
10 (0.555 g). The suspension was poured onto a piece of aluminum foil to cool and ground in the mill as described previously to collect all the particles <250 microns. The homogeneity of the matrix was checked by sampling out 200 mg of the matrix, extracting it with
15 10 mM PBS (pH: 7.4), and analyzing the solution for protein concentration. The matrix was then compressed as described before.

"Melt-cast" slabs were prepared with candelilla wax and 2% Macol® CA-20 using the procedure given for the
20 beeswax slabs above. Strips were cut from the slab with a hot razor blade because of the hard, brittle nature of the candelilla wax. The weights and surface areas of the devices from the three techniques were similar.

25 Density of Compressed Disks and Cast Slabs: The true density of the disks were determined with a helium pycnometer. The bulk volumes of the compressed disks were calculated from their dimensions. The bulk
30 volumes of the melt-cast slabs were determined with a pycnometer by displacement of an ethanol/water mixture of known density. The porosities of the compressed disks and cast slabs were calculated from their true volume and bulk volume.

In vitro release profiles were conducted on slabs by placing them into screw cap culture tubes (125 mm x 20 mm) which were previously autoclaved, dried, and filled with 8 ml of 10 mM PBS (pH: 7.4). Screw-cap
5 Erlenmeyer flasks (50ml) were used for the compressed disks. Gentamycin sulfate (100 ppm) was added to the buffer as an antimicrobial agent. The tubes were shaken (200 rpm) in a water bath at 37°C. The solutions were decanted and replaced with fresh buffer
10 first after 8 hours and thereafter at 24 hour intervals. Eight implants were used for each system for all studies.

The absorbance of release solutions (previously filtered through 0.22 microns) was measured at 276
15 nanometers (nm) for BSA and 281 nm for lysozyme. The absorbance at 320 nm was subtracted from the 276 nm and 281 nm values to correct for random scattering. The concentrations of the protein solutions were determined by using the following absorptivities: BSA: $0.595 \text{ mg}^{-1} \text{ ml cm}^{-1}$
20 and lysozyme: $2.41 \text{ mg}^{-1} \text{ ml cm}^{-1}$.

Activities of lysozyme samples were analyzed by measuring the change in absorbance of a suspension of micrococcus lysodekticus substrate.

Example 1

25 Ten (10) trials comprising thirty-one (31) sets of compositions having different combinations of BSA, waxes, and surfactants were evaluated using the materials and methods described above. The compositions are shown in Table 2. The results are
30 shown in Figures 1 and 2.

Referring to Figures 1 and 2, the water-insoluble surfactant Mazol® dramatically increased the amount of protein released from beeswax pellets when compared to

the water-soluble surfactant PPG. Beeswax-PPG (10%) compositions released only about 25% of the protein after 10 days; beeswax-Mazol (10%) compositions released almost 100% of the protein after 14 days.

- 5 Indeed, beeswax-Mazol (2%) compositions release about twice as much protein (about 60%) as do the beeswax-PPG (10%) compositions, both compared after 14 days.

~~Example 2~~

Effects of BSA loading and water-soluble additives:

- 10 The formation of channels/pores in a solid matrix can be enhanced either by increasing the loading of the protein in the matrix or by incorporating other water-soluble additives. In both cases, the transport of the protein is made easier giving rise to high
15 release rates. This point was tested by preparing slabs containing 20% BSA (Set 9) and slabs containing 10% BSA along with 10% pentaerythritol (Set 10) and testing the release profiles as described previously. The results are shown in Figure 3.

- 20 Referring to Figure 3, both sets 9 and 10 showed similar release profiles and displayed higher release-rates than sets with 10% protein loading (Sets 6 and 11). These data suggest that the release-rates are affected by the type of additive as well as its
25 concentration in the matrix. Comparing Figures 1 and 2, it is clear that high release rates of BSA can be obtained by: (1) increasing the loading of the protein; (2) incorporating large amounts of water soluble additives such as pentaerythritol; and (3)
30 incorporating a small amount of water-insoluble surfactant such as Mazol. The effectiveness of Mazol may be attributed to its water-insoluble, surface-active nature which can promote the permeation

of water into the matrix. PPG, on the other hand, is not surface-active, but is water-soluble. The difference between Mazol and PPG was also evident visually during the release study. Slabs containing
5 Mazol became white and slightly swollen, but those containing PPG did not.

Example 3

Effect of different surfactants as additive: In order to test the effect of different surfactants on
10 the release of BSA, slabs were prepared using surfactants with different HLB (Hydrophile Lipophile balance) numbers. A higher HLB number means the material is more hydrophilic. Table 1 lists the HLB numbers of the different surfactants used. The
15 effectiveness of these additives was compared at a 2% level in the matrix as previously described. The release profiles for these sets are shown in Figure 4.

Referring to Figure 4, there were minor differences in the release profiles for Mazol and Polysorbate 61,
20 but surprisingly there was no difference between Macol® CA-2 and Macol CA-20. This could have arisen from the differences in the molecular weights of Macol CA-2 and Macol CA-20 (about 350 daltons and about 1200 daltons respectively). Thus, the molar concentration of Macol
25 CA-2 in the wax was higher than that of Macol CA-20.

Example 4

Release of Lysozyme from Wax Slabs: During the preparation of the wax/protein slabs, the protein had to be kept at 85°C for about 2 minutes in the molten
30 wax. As these conditions could inactivate the protein, it became necessary to use a protein whose activity could be readily measured. Lysozyme was chosen for

this purpose and was released from slabs containing 0.0178 molal Macol CA-2 and 0.0178 molal Macol CA-20. The release profile of the matrix without any surfactant was similar to that with Macol CA-2 (Sets 24 and 25; Figure 5). The slab containing Macol CA-20 (Set 26) released substantially more of the lysozyme. The cumulative amount of lysozyme released, however, was much less than in the case of BSA. At the end of the release study, two slabs from Set 24 were extracted first with PBS and then with 50 mM glycine buffer (pH: 3.0). The total recovery from PBS and glycine buffers was only about 60%, with the balance being unaccounted. In contrast, all the protein could be accounted for in the experiments involving BSA. These results illustrate the intrinsic differences between these two proteins.

Release studies with lysozyme were stopped after 20 days and the residual protein was extracted from the wax slabs for measurement of bioactivity. Activity assays were performed on the following four lysozyme samples: (i) as received; (ii) purified; (iii) lysozyme extracted from a freshly cast slab and (iv) lysozyme recovered from the slabs at the end of the release study. The results are shown in Table 3.

Referring to Table 3, there was no difference in the activity of the four samples indicating that neither the formulation technique nor the release conditions had affected the bioactivity.

Example 5

A device containing BSA in a 1:1 mixture of beeswax and Stearine was formulated for testing as described previously. This composition also contained Macol CA-20 at 2% by weight (0.0178 molal). The composition

was tested as previously described for release characteristics. The results are shown graphically in Figure 6.

Referring to Figure 6, release of BSA was more rapid from the Stearine/beeswax matrix than from the beeswax matrix (Set 20).

Example 6

Comparison of Cast Wax Slabs and Compressed Wax Disks: As discussed in the Background, there is prior art relating to the sustained release of low molecular weight organic drugs from wax matrices. In almost all the cases cited, the drug was dispersed or dissolved in molten wax. The matrix was then crushed, sized, and tableted. Sustained release of the drug was obtained from these devices over a period of several hours (usually less than 24 hours). The presence of surfactants in the matrix influenced the release rate of the drug. Other significant variables were the particle size before tableting and the drug loading.

The role of the formulation technique in prolonging the duration of release was determined. Three techniques were compared in their effectiveness to achieve sustained release of BSA: (a) "Melt-cast slab" (Set 29); (b) "Dry-mixed compressed disk" (Set 30), and (c) "Melt-mixed compressed disk" (Set 31).

Compressed disks and cast slabs were prepared as previously described and tested for release profile. Candelilla wax was used in all the cases because of its low melting point (65-70°C) and ease of tableting. The surface areas of the disks and the slabs were similar (3.31 sq. cm vs 3.25 sq. cm). The loading of BSA was 10% w/w for all disks and slabs tested. The porosities of the compressed disks and cast-slabs were calculated

using the equation: $E = 100(1 - (v_t/v_b))$, where v_t = true volume and v_b = bulk volume of the matrix, respectively. The true volume and bulk volume were determined as described previously. The results are shown in Figure 7.

Referring to Figure 7, release of BSA was much faster from the compressed disks than from the cast slab. The "dry-mixed" and "melt-mixed" compressed disks (Sets 30 and 31 respectively) showed similar release profiles and released 100% in about 3 days. The "melt-mixed" disks, however, gave a slower release rate than the "dry-mixed" disks in the first 24 hours of the study. In the "melt-mixed" technique, the protein was added to the molten wax.

Example 7

A mixture of Zn-rpST/L-Arginine (1:1) was formulated into slabs containing Beeswax (100%) or Beeswax/Mazol (90:10). The compositions were prepared and tested as described above. From the results shown in Table 4, it is evident that PST release is higher from wax slabs containing Mazol than from those without Mazol.

Obviously many modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims the invention may be practiced otherwise than as specifically described.

Table 1

| Properties of Surfactants Used | | | | |
|----------------------------------------------------------------------------------------------------|----------------------------------------------------------------|-------------------------------|------------------|--|
| Surfactant | Chemical Classification | Molecular Weight ¹ | HLB ² | |
| Mazol® 80 MG-K | Ethoxylated mono- and di-glycerides | 1400 | 13.5 | |
| Macol® CA-2 | Polyoxyethylene ether of cetyl alcohol (2 units of EO) | 30 | 4.9 | |
| Macol® CA-20 | Polyoxyethylene ether of cetyl alcohol (20 units of EO) | 1122 | 15.7 | |
| T-MAZ®-61 | Polyoxyethylene ether of sorbitan monostearate (4 units of EO) | 706 | 9.5 | |
| ¹ In daltons ² Hydrophile Lipophile Balance number EO = ethylene oxide | | | | |

Table 2

5

Compositions of the Systems Evaluated

| 10 | ----- | | | |
|-------|----------|--------|------------|--------------------------------------|
| | Set # | Code | Trial # | Description |
| ----- | | | | |
| | 6 | BW | 3 | Beeswax/BSA 90/10 |
| | 7 | BW2P | 3 | Beeswax/PPG/BSA 90/2/10 |
| | 8 | BW10P | 3 | Beeswax/PPG/BSA 90/10/10 |
| 15 | 9 | BWBSA | 3 | Beeswax/BSA 80/20 |
| | 10 | BWBPE | 3 | Beeswax/Pentaerythritol/BSA 80/10/10 |
| | 11 | BW | 4 | Beeswax/BSA 90/10 |
| | 12 | BW2M | 4 | Beeswax/Mazol/BSA 90/2/10 |
| | 13 | BW10M | 4 | Beeswax/Mazol/BSA 90/10/10 |
| 20 | 17 | CA2/2 | 6 | Beeswax/Macol CA2/BSA 90/2/10 |
| | 18 | PSOR/2 | 6 | Beeswax/Polysorbate/BSA 90/2/10 |
| | 19 | MAZ/2 | 6 | Beeswax/Mazol/BSA 90/2/10 |
| | 20 | CA20/2 | 6 | Beeswax/Macol CA20/BSA 90/2/10 |
| | 24 | BW | 8 | Beeswax/Lysozyme 90/10 |
| 25 | 25 | BWC2 | 8 | Beeswax/Macol CA2/Lysozyme 90/.59/10 |
| | 26 | BWC20 | 8 | Beeswax/Macol CA20/Lysozyme 90/2/10 |
| | 28 | STBW | 10 | Stearin/Beeswax/CA-20/BSA 45/45/2/10 |
| | 29 | CNCS | 10 | Candelilla Wax/CA-20/BSA 90/2/10 |
| | | | | Melt cast slab |
| 30 | 30 | CNDM | 10 | Candelilla Wax/CA-20/BSA 90/2/10 |
| | | | | Compressed disk; dry mixed |
| | 31 | CNMC | 10 | Candelilla Wax/CA-20/BSA 90/2/10 |
| | | | | Compressed disk; melt mixed |
| | ----- | | | |

Table 3

| Activity of Lysozyme Recovered From the Wax Formulations | | |
|-------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| | Sample | Activity (10^{-4}) ¹ |
| | Starting Material ² | 6.3 ± 0.6 |
| 10 | BWC20/EXT ³ | 8.5 ± 0.7 |
| | Set 24 ⁴ | 6.5 ± 0.6 |
| | Set 25 ⁴ | 5.2 ± 0.2 |
| | Set 26 ⁴ | 6.4 ± 0.5 |
| 15 | ¹ units/mg protein (Average of 5 replicates) ² purified lysozyme used in slabs ³ extracted from unreleased slab ⁴ one sample from each set was used | |

Table 4

| Release of PST from Beeswax Slabs with and without Mazol | | |
|-------------------------------------------------------------|----------------|-----------------------------------------------|
| | Time (Days) | Cumulative Percent Released |
| | | Beeswax without Mazol Beeswax with Mazol |
| | 0.34 | 5.8 ± 1.4 10.8 ± 0.29 |
| | 1.00 | 7.1 ± 1.3 16.3 ± 0.37 |
| | 2.00 | 7.6 ± 1.4 18.7 ± 0.36 |
| 35 | 3.00 | 8.1 ± 1.5 19.9 ± 0.42 |
| | 4.00 | 8.8 ± 1.5 29.3 ± 0.47 |
| | 5.00 | 9.2 ± 1.4 20.6 ± 0.54 |
| | 6.00 | 9.5 ± 1.4 20.9 ± 0.57 |
| 40 | 7.00 | 9.8 ± 1.4 21.1 ± 0.62 |

WHAT IS CLAIMED IS:

1. A sustained release composition for delivering macromolecular proteins to an animal over a prolonged
5 period, comprising:
a solid wax matrix;
a macromolecular protein uniformly dispersed in said wax matrix; and
a water-insoluble surfactant uniformly dispersed in
10 said wax matrix.
2. The sustained release composition of Claim 1 wherein said wax comprises from about 50-99% by weight of said composition, said protein comprises from about 1-30% by weight of said composition, and said
15 surfactant comprises from about 0.1-20% by weight of said composition.
3. The sustained release composition of Claim 1 wherein said wax is selected from the group consisting of animal waxes, vegetable waxes, mineral waxes, and
20 petroleum waxes, or combinations thereof.
4. The sustained release composition of Claim 1 wherein said wax is selected from the group consisting of beeswax, lanolin, shellac wax, Chinese insect wax, hydrogenated soybean oil, cottonseed oil, carnauba,
25 candelilla, bayberry, sugar cane, fossil or earth waxes (ozocerite, ceresin, montan) and paraffin, microcrystalline, slack and scale wax, or combinations thereof.
5. The sustained release composition of Claim 1
30 wherein said surfactant is selected from the group consisting of surfactants having a molecular weight (MW) of from about 100-2000 and a Hydrophile Lipophile balance (HLB) value of from about 1-17.

6. The sustained release composition of Claim 1 wherein said surfactant is selected from the group consisting of Mazol®, Macol® CA-2, Macol® CA-20 and T-MAZ®-61.

5 7. The sustained release composition of Claim 1 wherein said surfactant is Mazol®.

8. The sustained release composition of Claim 1 wherein said macromolecular proteins are selected from the group consisting of enzymes, enzyme inhibitors,
10 antibodies, antigens, interferons, insulins, prolactins, somatomedins, somatostatins, interleukins, somatocrinins (GRF) and somatotropins.

9. The sustained release composition of Claim 1 wherein said macromolecular protein is a somatotropin.

15 10. The sustained release composition of Claim 9 wherein said somatotropin is selected from the group consisting of human, bovine, ovine, avian, equine, and porcine somatotropins.

11. The sustained release composition of Claim 10
20 wherein said somatotropin is porcine somatotropin.

12. The sustained release composition of Claim 9 wherein said wax is beeswax and said surfactant is Mazol®.

13. The sustained release composition of Claim 12
25 comprising from about 50-99% by weight beeswax, from about 1-30% by weight somatotropin, and from about 0.1-20% by weight Mazol®.

14. A method for delivering macromolecular
30 proteins to an animal over a prolonged period, comprising:

administering to said animal the composition of Claim 1.

15. The method of Claim 14 wherein said wax comprises from about 50-99% by weight of said

composition, said protein comprises from about 1-30% by weight of said composition, and said surfactant comprises from about 0.1-20% by weight of said composition.

5 16. The method of Claim 14 wherein said wax is selected from the group consisting of animal waxes, vegetable waxes, mineral waxes, and petroleum waxes, or combinations thereof.

10 17. The method of Claim 14 wherein said wax is selected from the group consisting of beeswax, lanolin, shellac wax, Chinese insect wax, hydrogenated soybean oil, cottonseed oil, carnauba, candelilla, bayberry, sugar cane, fossil or earth waxes (ozocerite, ceresin, montan) and paraffin, microcrystalline, slack and scale
15 wax, or combinations thereof.

18. The method of Claim 14 wherein said surfactant is selected from the group consisting of surfactants having a molecular weight (MW) of from about 100-2000 and a Hydrophile Lipophile balance (HLB) value of from
20 about 1-17.

19. The method of Claim 14 wherein said surfactant is selected from the group consisting of Mazol®, Macol® CA-2, Macol® CA-20 and T-MAZ®-61.

20 20. The method of Claim 14 wherein said surfactant is Mazol®.

21. The method of Claim 14 wherein said macromolecular proteins are selected from the group consisting of enzymes, enzyme inhibitors, antibodies, antigens, interferons, insulins, prolactins, somatomedins, somatostatins, interleukins, somatocrinins (GRF) and somatotropins.
30

22. The method of Claim 14 wherein said macromolecular protein is a somatotropin.

23. The method of Claim 22 wherein said somatotropin is selected from the group consisting of human, bovine; ovine, avian, equine, and porcine somatotropins.

5 24. The method of Claim 23 wherein said somatotropin is porcine somatotropin.

25. The method of Claim 22 wherein said wax is beeswax and said surfactant is Mazol®.

10 26. The method of Claim 25 comprising from about 50-99% by weight beeswax, from about 1-30% by weight somatotropin, and from about 0.1-20% by weight Mazol®.

27. A method for promoting growth and increasing feed utilization efficiency for an animal, comprising; administering to said animal the composition of
15 Claim 9.

28. The method of Claim 27 wherein said somatotropin is selected from the group consisting of human, bovine, ovine, avian, equine, and porcine somatotropins.

20 29. The method of Claim 28 wherein said somatotropin is porcine somatotropin.

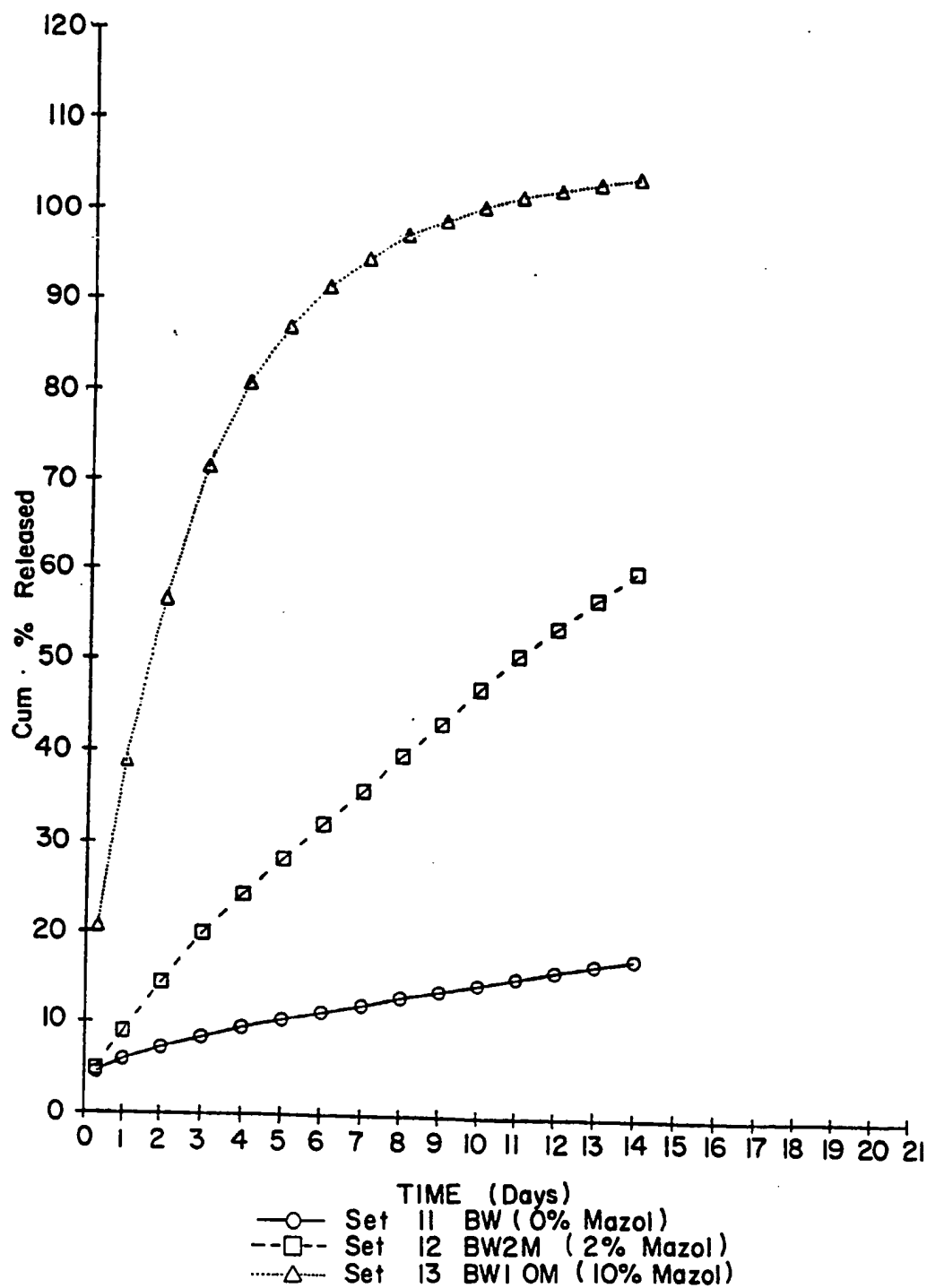
30. The method of Claim 27 wherein said wax is beeswax and said surfactant is Mazol®.

25 31. The method of Claim 30 comprising from about 50-99% by weight beeswax, from about 1-30% by weight somatotropin, and from about 0.1-20% by weight Mazol®.

32. The method of Claim 27 wherein said wax comprises from about 50-99% by weight of said composition, said somatotropin comprises from about
30 1-30% by weight of said composition, and said surfactant comprises from about 0.1-20% by weight of said composition.

1 / 7

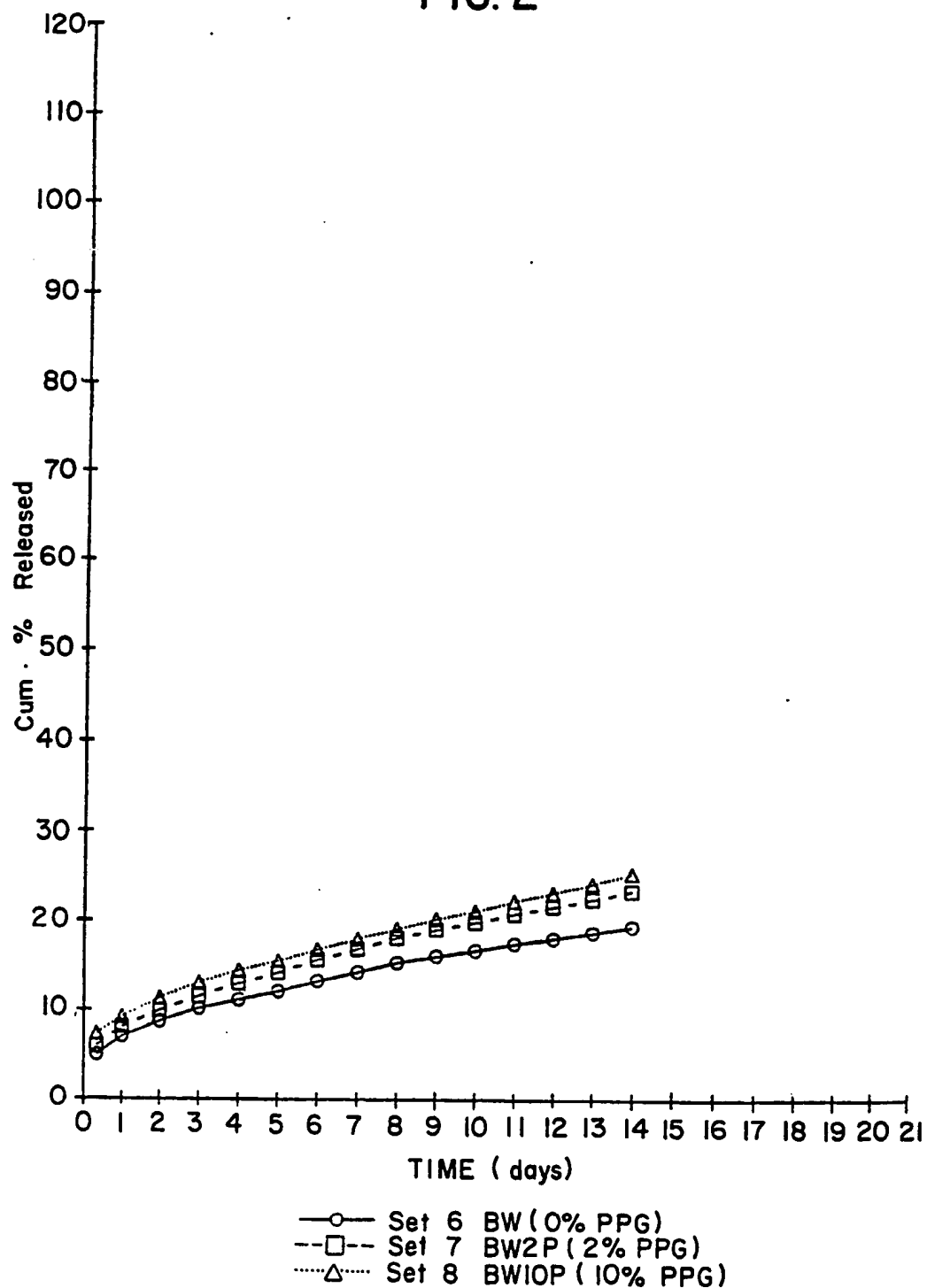
FIG. 1



SUBSTITUTE SHEET

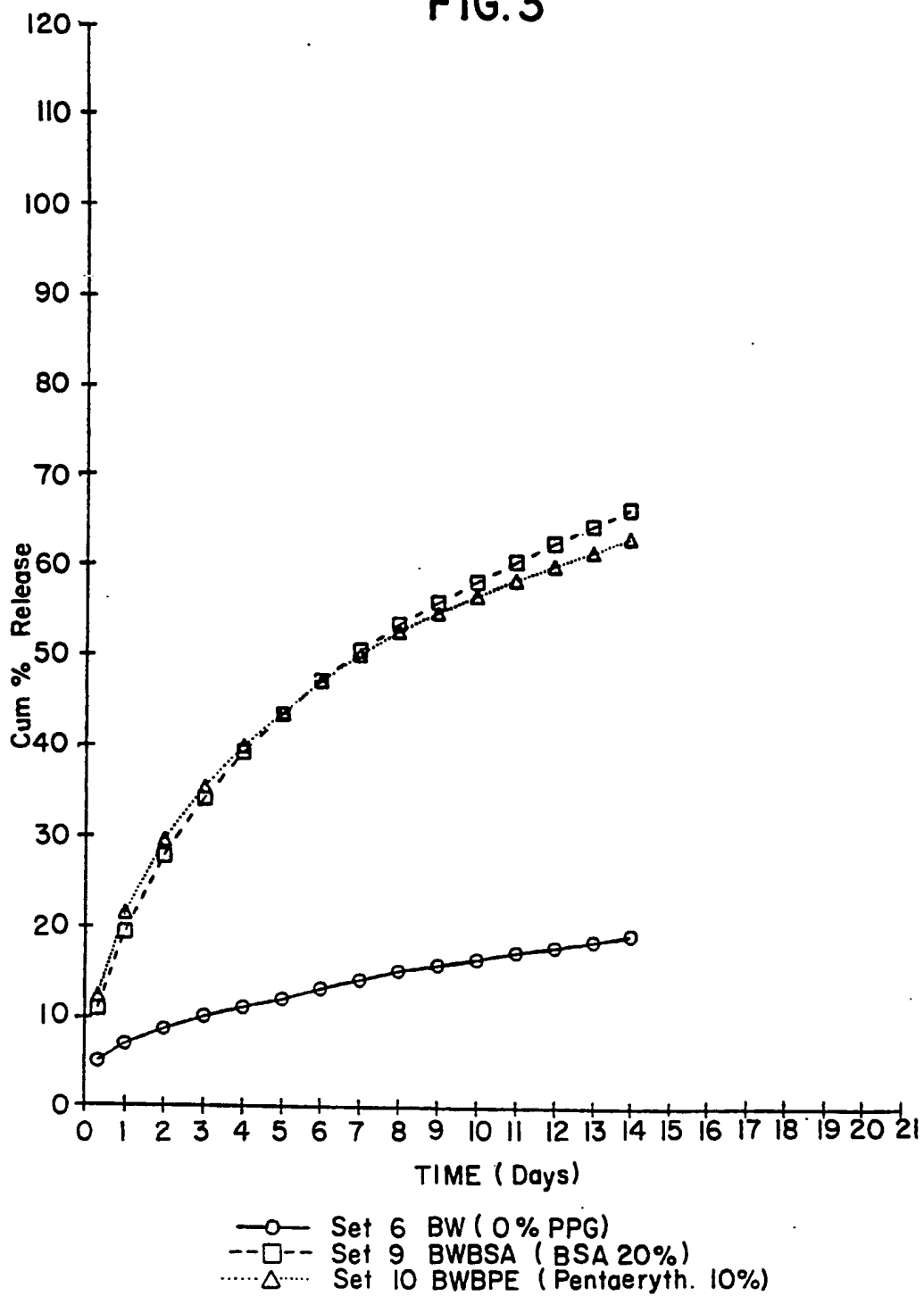
2 / 7

FIG. 2



3 / 7

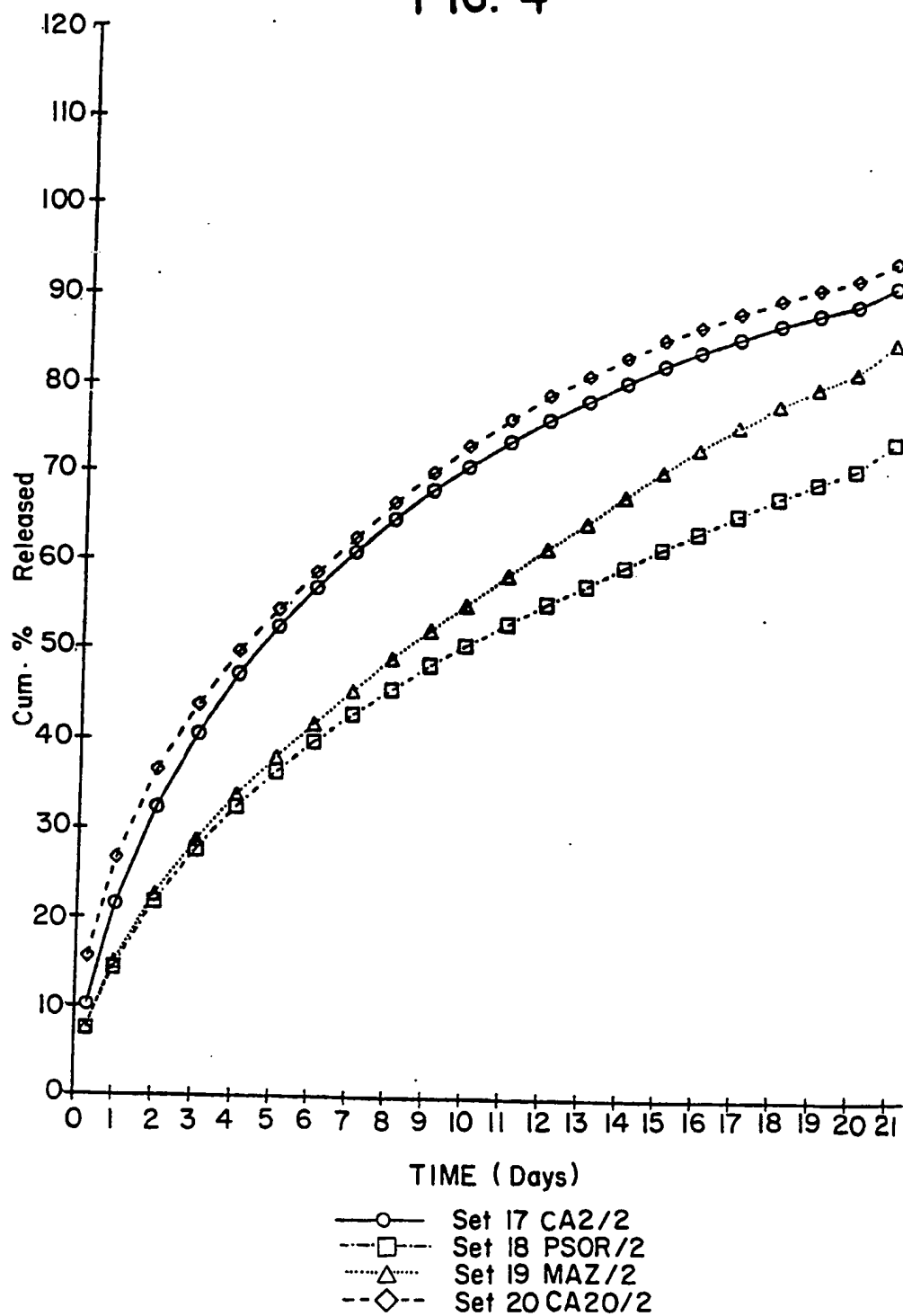
FIG. 3



SUBSTITUTE SHEET

4 / 7

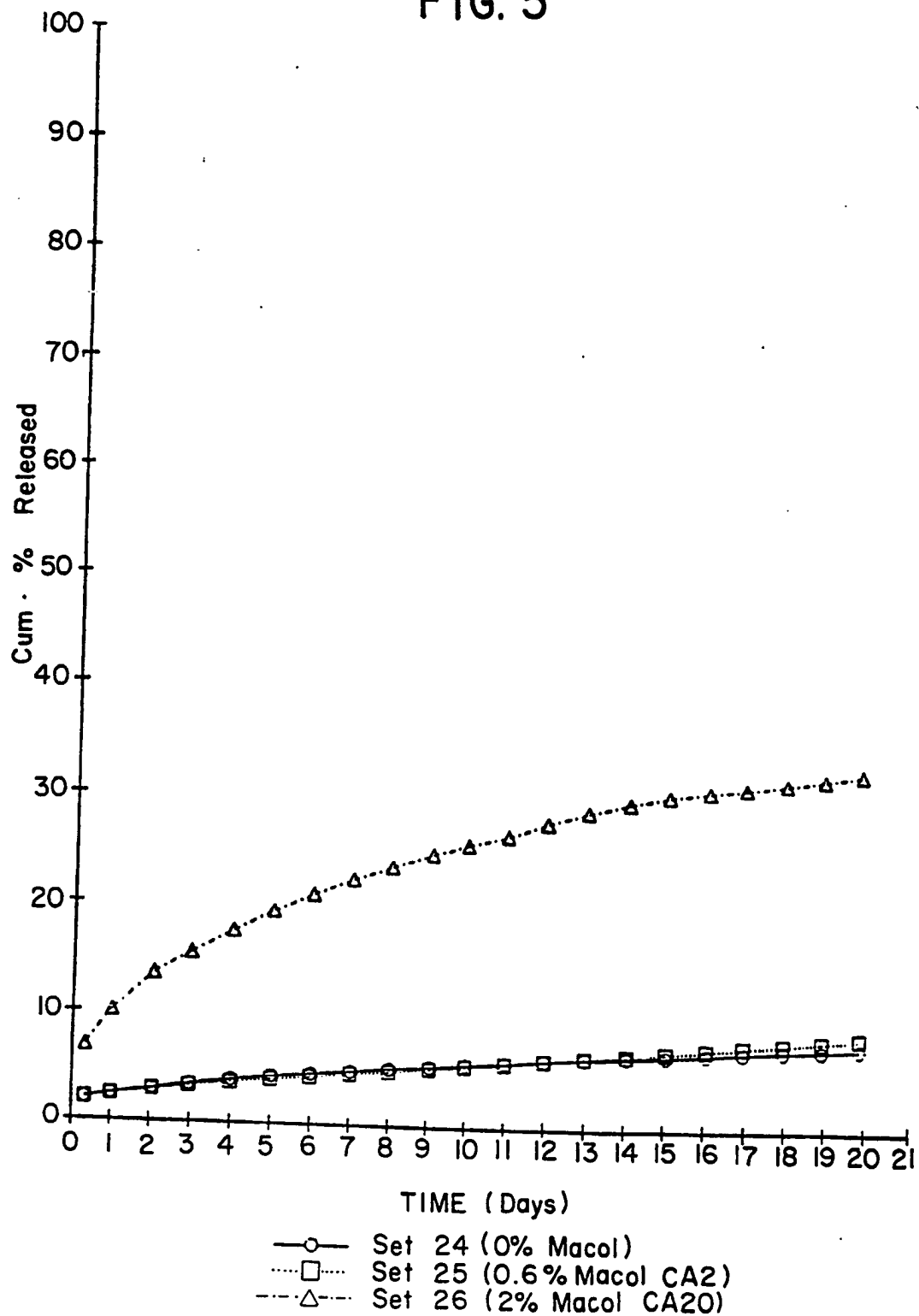
FIG. 4



SUBSTITUTE SHEET

5/7

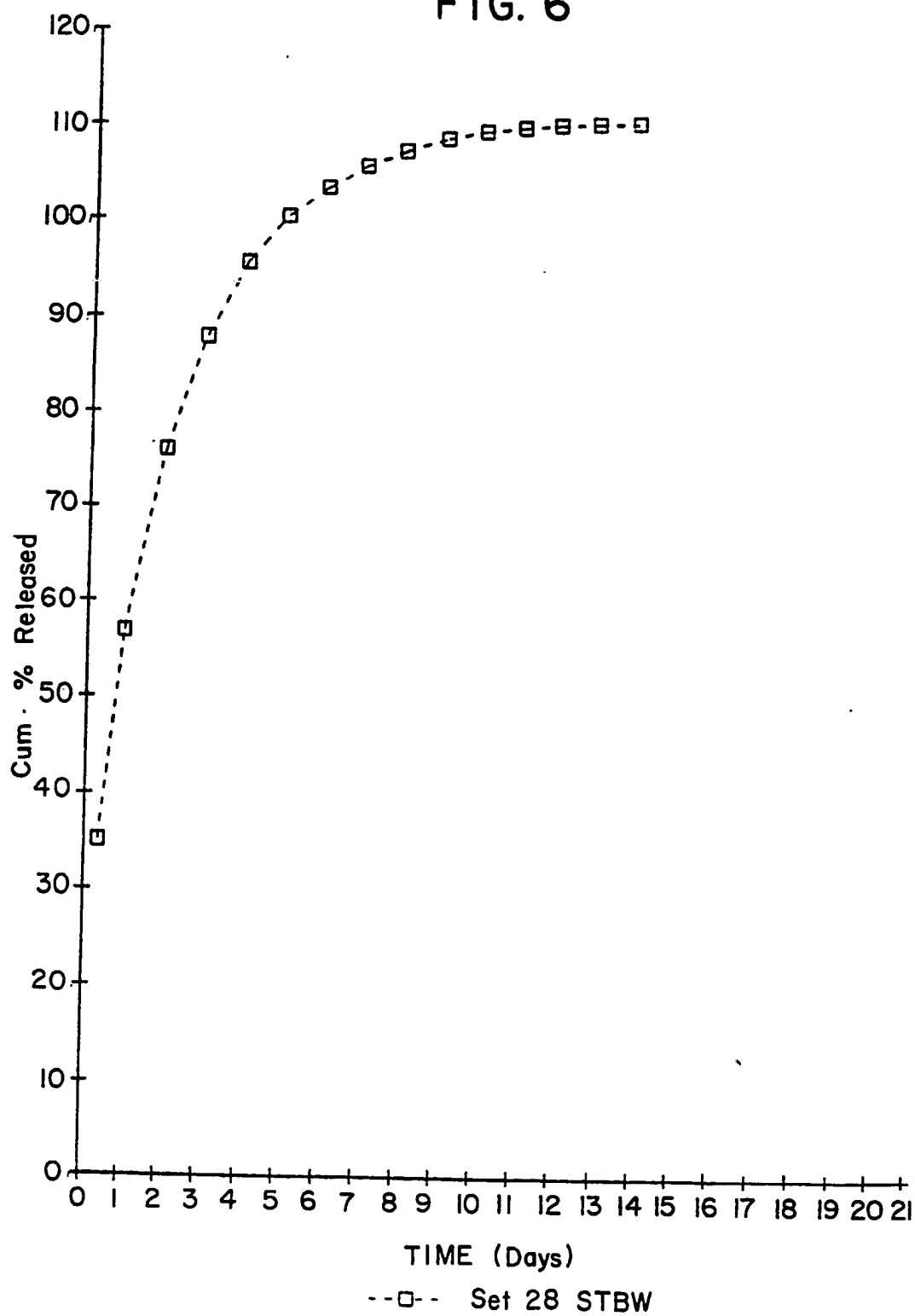
FIG. 5



SUBSTITUTE SHEET

6 / 7

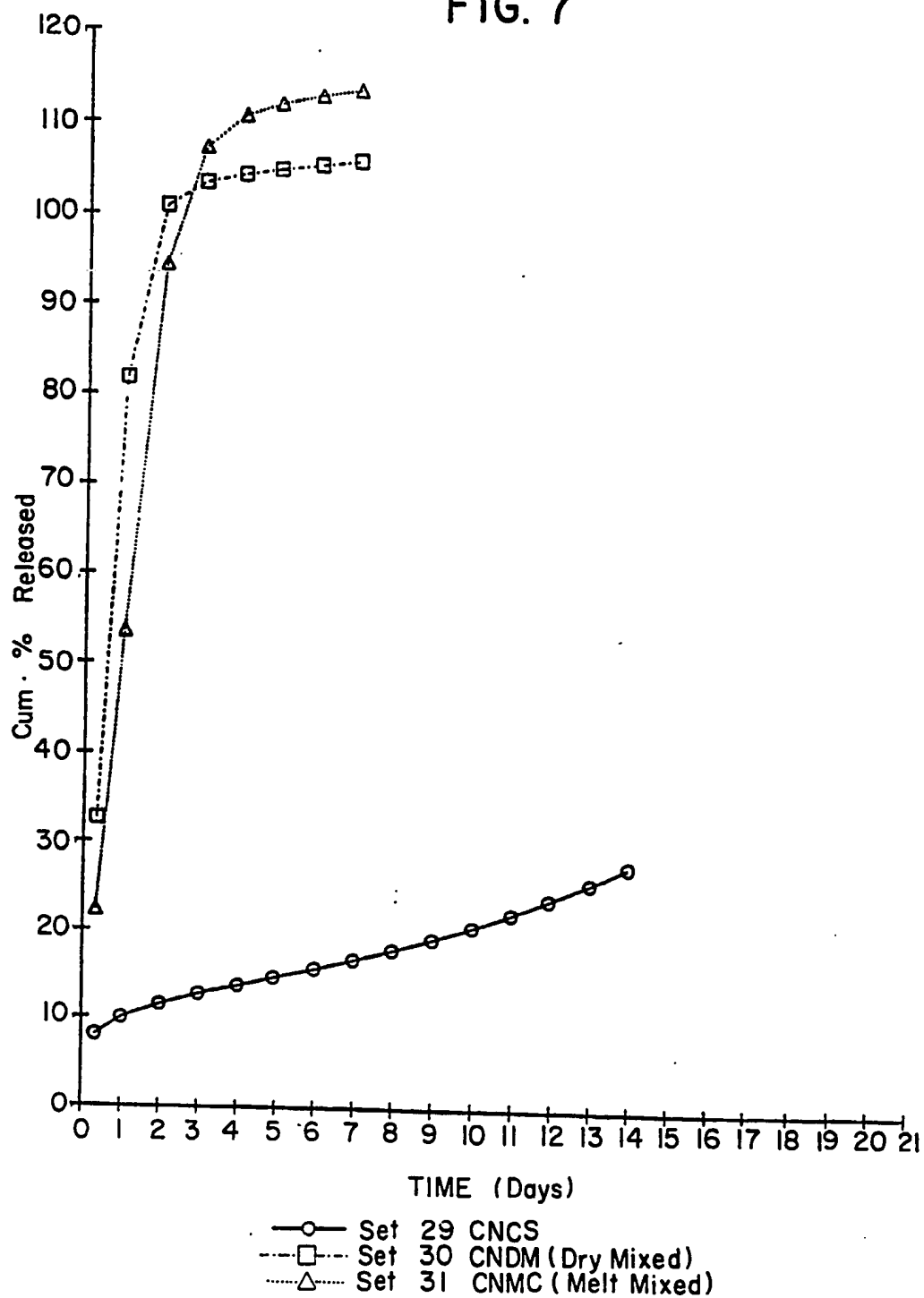
FIG. 6



SUBSTITUTE SHEET

7/7

FIG. 7



INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/05345

| | | |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶ | | |
| According to International Patent Classification (IPC) or to both National Classification and IPC | | |
| IPC ⁵ : A 61 K 9/22 | | |
| II. FIELDS SEARCHED | | |
| Minimum Documentation Searched ⁷ | | |
| Classification System | Classification Symbols | |
| IPC ⁵ | A 61 K | |
| Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸ | | |
| | | |
| III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹ | | |
| Category ¹⁰ | Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹² | Relevant to Claim No. ¹³ |
| X | EP, A, 0257368 (AMERICAN CYANAMID COMPANY) 2 March 1988 see page 2, line 1 - page 12, end; claims -- | 1-5,8-11 |
| X | US, A, 2413419 (SAUNDERS et al.) 31 December 1946 see the whole document, in particular column 5, line 58 -- | 1,4,5 |
| A | EP, A, 0246540 (WANG) 25 November 1987 ----- | |
| <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁴ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"A" document member of the same patent family</p> </div> </div> | | |
| IV. CERTIFICATION | | |
| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report | |
| 7th January 1991 | 24. 01. 91 | |
| International Searching Authority | Signature of Authorized Officer | |
| EUROPEAN PATENT OFFICE | <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="border: 1px solid black; padding: 2px 10px;">M. PEIS</div> <div style="text-align: right;">M. 023</div> </div> | |

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 14-32 because they relate to subject matter not required to be searched by this Authority, namely:

Pls. see Rule 39.1 (iv) - PCT:

Method for treatment of the human or animal body by surgery or therapy,
as well as diagnostic method.

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers _____, because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9005345
SA 40176

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 21/01/91. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|-------------------------------------------|---------------------|----------------------------|---------------------|
| EP-A- 0257368 | 02-03-88 | AU-B- 597708 | 07-06-90 |
| | | AU-A- 7672587 | 18-02-88 |
| | | JP-A- 63048223 | 29-02-88 |
| | | US-A- 4837381 | 06-06-89 |
| | | ZA-A- 8705898 | 12-02-88 |
| ----- | | | |
| US-A- 2413419 | | None | |
| ----- | | | |
| EP-A- 0246540 | 25-11-87 | CA-A- 1257199 | 11-07-89 |
| | | JP-A- 63022012 | 29-01-88 |
| ----- | | | |